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Effect of Krill Oil Application on Wound Healing in Male Rats

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Abstract: ***Wound healing is an active process of communication between cells, matrix, and various growth factors. Krill oil is a rarely used substance having medicinal properties with wound healing, as anti-inflammatory, antioxidant and renewal of tissues. The current study aimed to study the role of krill oil in the wounded skin healing and repair. Skin wound incisions were performed in the right thigh area of thirty mature male rats (aged 10 weeks and weighing 165±4.5 g). Wounded skin rats were assigned into two equal groups. Wounded areas were daily treated with a local application of 1 µL of normal saline (control group) and 1 µL of krill oil (treatment group). After 1, 5, and 10 days, five rats from each group were sacrificed and tissue samples from the wounded areas were obtained for histopathological examination. Histopathological examination revealed time-dependent successive acceleration of wound healing in the krill oil-treated rats compared with that of control rats throughout the experimental period. Improvements were observed with regard to the level of epithelialization, infiltration of inflammatory cells, and constriction of the wound area. In conclusion, local application of krill oil has therapeutic efficiency to accelerate wound healing.***

Keywords: Krill oil, Wound healing, Growth factors

1. Introduction

In addition the protective mechanism, the skin possesses various functions, where it serves as a mechanical barrier between the inner and external parts of the body [1]. From outside, the skin consists of the epidermis, dermis, and the hypodermis [2]. In general a skin wound was defined as damage caused by physical and chemical agents or result from the presence of medical or physical condition in the underlying tissues [3]**.** The four steps of wound healing (hemostasis, inflammation, proliferation, and remodeling) are regulated by various factors including cytokines and growth factors released by cells in the wounded area. For acute wound, the phases are linear and overlapping, whereas can be distinguished in chronic wounds, where the steps of wound healing can be found at different stages [4]**.**

Krill oil (KO) is extracted from the largest of the krill species, the Antarctic *Euphausia superba*. The medicinal importance of krill oil is attributed to its anti-inflammatory and antioxidant potency as well as its role in the improvement of brain function and protection of cardiovascular system. It has been found that KO is rich in a natural antioxidant, astaxanthin, which has powerful protective role of different cells from free radical damage caused by overexposure to UV sunlight and chemical pollutants [5], and improves skin condition including reducing crow's feet type wrinkles and increasing skin moisture content [6].

Krill is a rich source of omega-3 and omega-6 polyunsaturated fatty acids (*n*-3 and *n-6* PUFAs), especially the long-chain PUFAs eicosapentaenoic acid (EPA; C20:5 *n*-3) and docosahexaenoic acid (DHA; C22:6 *n*-3). These fatty acids (FAs) were found to be bound in phospholipids (PLs) with phosphatidylcholine (PC) being the most abundant form [5]. Phospholipid omega-3 fatty acids (FAs) keep cell membranes flexible, fluid, and healthy, including those in skin cells [6].

The present study aimed to examine the protective ability of topically applied KO to modulate wound healing in a rat model of skin wounding.

2. Material and Methods

Experimental animals: The current study was carried out at the College of Dentistry, University of Baghdad during the period extended from 5 January 2017 to 20 March 2017. All experimental procedures were conducted according to the ethical guidelines and policies of Baghdad University, Iraq. Albino male rats were obtained from the animal house of the College of Veterinary Medicine, University of Al-Qadisiyah, Iraq. Thirty males, weighed 165±4.5 g and aged 10 weeks, were housed individually in plastic cages (30 × 15 × 12 cm) with a stainless-steel buckle under controlled conditions (12:12 light and dark cycle and 22–24 °C temperature), and fed on standard laboratory chow (19% protein and 3000 kilocalories energy) and drinking water *ad libitum*.

Experimental groups: The rats were subjected to skin incision under aseptic conditions. Each rat was anaesthetized by intramuscular injection of xylazine (0.4 mg/kg body weight) and ketamine HCl (40 mg/kg body weight). A skin incision was performed on the right side of the thigh, and the area was air-dried. Incision area was locally applied daily with 1 µL of normal saline (control group) and 1 µL of KO (treatment group). After 1, 5, and 10 days, five males from each group were sacrificed and samples from the wound area were excised and fixed with 10% buffered formalin solution for histopathological examination.

Methods: Histological sections were prepared (5-6 µm in thickness), stained with haematoxylin and eosin according to that previously reported by Lee et al. [7], and then microscopically examined under 10 and 40 × magnification.

3. Results

At 1 Day duration: Histological findings (Hematoxylin and Eosin stain), after one day of skin incision, of control and krill oil treated group male rats showed the defect area, no epithelialization yet and migration of high numbers of epithelial cells with low granulation tissue (figure 1).

At 5 Days duration: The histological findings at the wound site of control group males showed epithelialization, decreased numbers of inflammatory cells, appearance of irregular loosely formed collagen fibers and the presence of new blood vessels with the formation of granulation tissue, (figure 2-C). The histological findings of the KO-treated rats showed a declined inflammatory cell number, replacement of granulation tissue with fibrous tissue and the presence of scattered fibroblasts, complete epithelialization, and formation of new blood vessels, condensed collagen fibers with signs of remodeling (figure 2-T).

At 10 Days duration: The histological sections obtained from the control group rats after 10 days of treatment showed epithelialization with a complete basal structure together with remodeling collagen fibers and formation of new blood vessels (figure 3-C). The histological sections from KO-treated male rats showed complete epithelialization with thin and no rete edges epithelium and presence of fibrous tissue and reduced cellular components (figure 3-T).

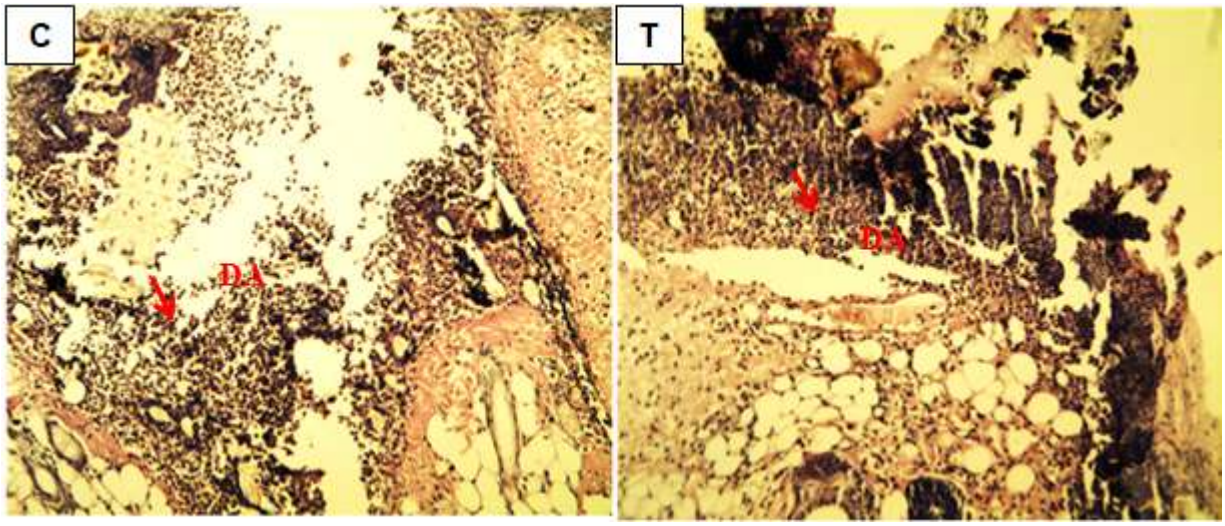

Figure 1: Histopathological evaluation of male rat skin wound from control (C group) and krill oil treated (T group) after 1 day shows defect area (DA) with infiltration of inflammatory cells (head arrow). H&E x10.

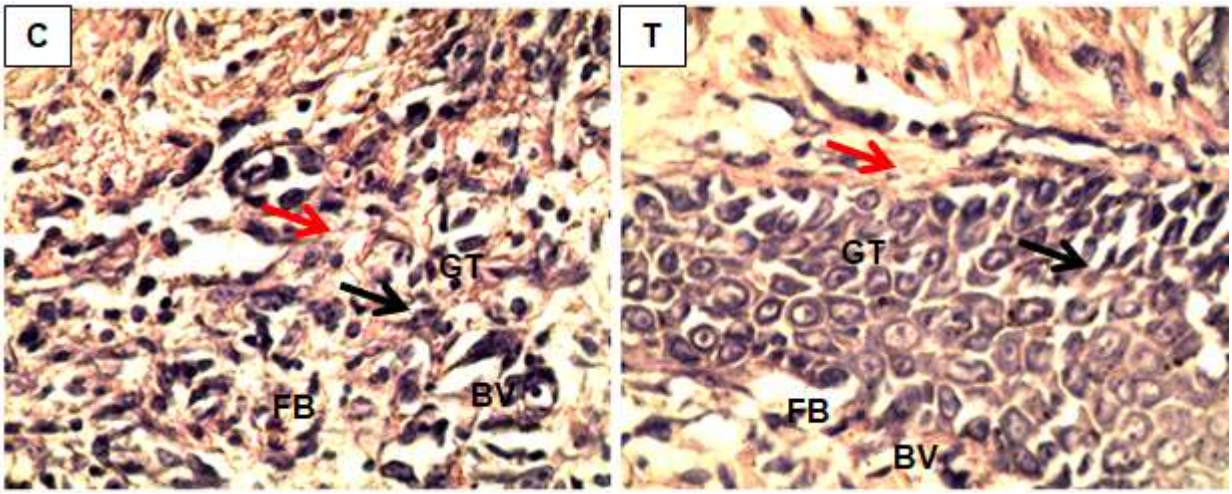

Figure (2): Histopathological evaluation of male rat skin wound after 5 day. C: Micrographs of wound site from control group male shows granulation tissue at defect area (GT), migrating epithelial cells (black arrow), collagen fibers (red arrow), new blood vessel (BV) and fibroblasts (FB). T: Micrographs of wound site from krill oil treated group shows migration of epithelial cells to the defect of area (black arrow), fibroblast (FB), collagen fibers (red arrow) and new blood vessels (BV). H&E x40.

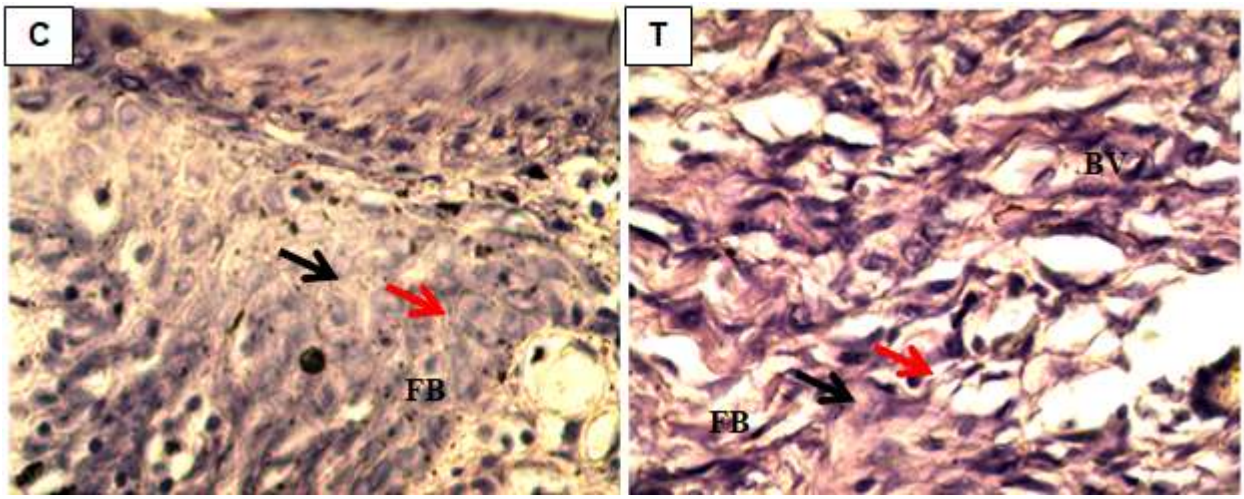

Figure 3: Histopathological evaluation of male rat skin wound after 10 days. C: Micrographs of wound site from control group shows complete epithelialization at the defect edge (black arrow), collagen fibers (red arrow), and fibroblasts (FB). T: Micrographs of wound site from krill oil treated group shows complete epithelialization at the defect edge (black arrow), fibroblasts (FB), new blood vessels (BV), and collagen fibers (red arrow). H&E x40.

4. Discussion

In the control group males at 1 day, histological findings revealed areas of granulation tissue formation, epithelial cell migration, and the presence of inflammatory cells. These findings agree with those of a previous study [8], where they found, after 4 days of skin wounding, migrating epithelial cells, and disappearance of the necrotic debris from the wound surface. Also they found presence of an inflammatory phase during days 5 and 10 after wounding. In our control group 5 days after the incisional wound, histological evaluation revealed complete epithelialization. This result agrees with that of Al-Wattar [9]. It has been mentioned that wound healing is a natural process to return the skin or mucosa back to its normal state [10].

In treatment group male rats, 5 days after the incisional wound, the histological findings showed declined inflammatory cell numbers and replacement of granulation tissue with fibrous connective tissue. After 10 days, histological findings revealed similar characteristic features among the experimental and control groups, characterized by the formation of new epithelium, active fibroblasts, and remodeling of collagen fibers and granulation tissue. This result agrees with that reported by another study [11]. In the current study, we selected time points of 1, 5, and 10 days to provide a unique perspective, because prior studies have frequently focused on durations of 2, 7, and 14 days.

The results of control group showed gradual decrease in epithelialization, whereas in treated group the process of wound surface epithelialization was enhanced and accelerated by topical krill oil application. Also in both groups, the epithelialization reached its peak at day 5 then declined 10 days and this result was in agreement with [9], which could be due to the remodeling process that associated with final stages of healing. Epithelialization is known as a process where epithelial cells (basal keratinocytes) arising from either the wound margins or residual dermal epithelial appendages within the wound bed with additional epithelial cells provided by the proliferation of immature keratinocytes in the basal layer begin to migrate over the underlying viable connective tissue [12]. Marginal basal cells at the edge of the wound lose their firm attachment to the underlying dermis, enlarge and begin to migrate across the surface [12,13].

During inflammation the blood clots are formed, the inflammatory cells reach the injured region and the keratinocytes migrate through the wound initiating re-epithelialization [14]. The ability of KO as anti-inflammatory is maintained when the damage is worsened by inflammation. Cells exposed to KO were able to heal much faster than those unexposed. These evidences emphasize a main function of KO in improving the healing of damaged epithelia. Similarly, KO was also shown to improve epithelial cell survival by reducing cell death during inflammation. This could be due to the presence of omega-3, since comparing of KO to another oil (corn oil) with a different lipids composition and completely lacking of omega-3, where corn oil was unable to counteract the effect of cytomix in inducing the pro-inflammatory cytokines, IL-8 and TNF-α [15].

The current results provide strong evidence to support the value of KO in down-regulating skin incision inflammation by inducing epithelial functional and morphological restitution, improving cell survival and reducing the adhesiveness. Krill oil can boost energy where it can switches on genes involved in the mitochondrial electron transport chain for producing cellular energy. This increased energy production effect has not been reported with fish oils [16].

In conclusion, the effect of KO in our study has provided firm evidence that topically applied KO can improve and accelerate skin healing. This agent shows promise; not only to ameliorate acute wound healing, but also to bring determination to chronic skin wounds that resist healing.

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